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A comparative assessment of serum, plasma and urine amylase levels in typhoid fever and HIV /AIDS patients

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ABSTRACT

The clinical use of Amylase enzyme activity as a diagnostic tool for pancreatic diseases has been well established but data on its use in other diseases such as typhoid fever and HIV/AIDS is scanty hence we carried out a Comparative assessment of the serum, plasma and Urine Amylase Levels (activities) in Typhoid fever and HIV /AIDS patients in order to ascertain its diagnostic value. Serum / plasma samples were collected from 206 subjects: 81 typhoid fever patients, 75 HIV infected and 50 normal subjects. Urine samples were also collected from all the subjects. The mean urinary amylase levels in typhoid fever, HIV infected and normal subjects were found to be higher than the mean serum or plasma amylase levels of the same subjects. The mean serum amylase levels of typhoid fever, HIV infected and normal subject were found to be almost the same with the mean plasma amylase levels of the subjects examined as both fell within the normal range. The mean serum plasma and urine amylase levels of typhoid fever and HIV infected subjects were found to be higher when compared with that of the normal subjects even though they all fell within the normal reference range. This result notwithstanding, showed that serum/plasma or urine amylase activity could be used as a diagnostic tool for typhoid fever and HIV/ AIDS patients if these tests are carried out as early as possible. It therefore recommended that more studies be done to ascertain the usefulness of Serum and urinary amylase analysis in differentiate between malaria and typhoid fevers both of which have similar clinical features.

Key words: Amylase level, HIV/ AIDS, typhoid fever, diagnostic tool

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INTRODUCTION

The enzyme amylase has a molecular weight of less than 50,000 Daltons. It is found in the salivary glands, exocrine pancreas and tissue specific isoenzymes. It can be distinguished by means of electrophoresis or the use of inhibitors [1]. Because amylase has molecular weight of less than 50,000 Daltons, it is readily excreted by the kidney in urine. Accordingly, the urinary excretion of Amylase is high in patients with acute pancreatitis and its presence reflects an increase in its activity in serum.

Measurement of urinary amylase may occasionally be of value in the diagnosis of short lived episodes of pancreatitis when the serum amylase is increased transiently. The increased excretion of amylase persists longer than the elevation in serum amylase activity and can help establish the diagnosis of acute pancreatitis. The urinary amylase may be elevated for 7 to 10 days whereas the serum amylase returns to normal in 2 or 3 days after an attack [1].

Amylases are secreted by the salivary and pancreatic glands into their respective juices, which enter the gastrointestinal tracts. These enzymes are important for the digestion of ingested starch but the amylase from the pancreas play the major role because the salivary amylase soon becomes inactive in the acidic environment of the stomach. Several Isoenzymes of both pancreatic and salivary amylases exist. The amylases that are normally present in serum are derived from both pancreas and salivary glands. The activity of serum amylase rises after an obstruction to the flow of fluid from either the salivary or the pancreatic glands but the elevation is usually much greater when the out flow from the pancreatic gland is blocked.

Acute pancreatitis is caused by blockage of the pancreatic ducts by direct injury to the pancreatic tissue by toxins, inflammation or trauma, or by impaired blood flow to the pancreas. The inflammation and autodigestion by pancreatic enzymes that accompany pancreatic injury usually result in an obstruction to the flow of pancreatic juice into the intestine. High levels of amylase activity may be found in pleural fluids in some cases of acute pancreatitis [1]. So far it has been established that increased serum amylase levels is a diagnostic tool for pancreatic disturbances but nothing has been said about serum amylase level with respect to typhoid fever and Human immuno deficiency Virus (HIV) disease [2, 3]. The aim of this research therefore is to asses the serum, plasma and urine amylase levels (activities) in typhoid fever and HIV/ AIDS patients and also to find out if the enzymes could be used as a diagnostic tool in clinical cases

MATERIALS AND METHODS

Materials used:
Test tubes, Racks, Centrifuge (Centromix P- selecta), Micropipettes (Transferpette) Micropipette tips. Water bath (Unitromic 6320100p-selecta) Spectrophotometer (Atom A- 390 BTR- 815) 10ml pipette, Measuring Cylinder, Heparinized and plain Containers

Reagents used:
Phosphate buffered starch substrate, Iodine solution, Distilled water, Blood, serum/plasma and Urine samples of typhoid fever, HIV infected and normal subjects.

Samples Assayed:
A total of 156 patients were studied: 81 typhoid fever patients, 75 HIV/AIDS patients and 50 normal subjects.

Assay of serum Amylase level.
Serum amylase analysis was carried out according to the caraway Amylase method as described by Hayakawa et al, 1973 [4].

Principle of Caraway Amylase method
Amylase is incubated at37^{o}C for exactly 71/2 minutes in an alkaline (PH) of 7.0 phosphate buffered medium.

Starch substrate: The enzyme hydrolysed the starch to maltose and other fragments. The amount of starch which remains unhydrolysed at the end of the incubation period reacts with iodine solution to give a violet blue black colour, the absorbance of which is measured in a spectrophotometer at 620mm wavelength. Enzyme activity is measured by the difference in absorbance of the starch-iodine complex of the sample against that of a reagent in which there is no hydrolysis.

Method (Procedure)
Blood collected in heparinized and plain containers were placed in a Centromix P- selecta centrifuge and then spun for 5 minutes to obtain the plasma and serum respectively

Specimen: The method required 0.01mL of patients serum or heparinized plasma that is free from haemolysis or urine
a. Six clean and dry test tubes were placed on a rack and one was labeled B i.e the Black, while others were labelles T i.e tests.
b. Using a Micropipette, O.4 mL of phosphate buffered starch substrates were withdrawn into each test tube respectively.

c. The contents of each test tube were mixed and placed in a water bath preincubated at 37°c for 5mins to warm the substrate.
d. 0.01 mL of sample was added to the test labeled tubes, mixed and incubated at 37°c for 71/2 mins
e. The tubes were removed from the water bath and 0.1m of working iodine solution was added to each tube.
f. 5ml of distilled water was added to each and mixed well
g. The absorbances were read of the tests and reagent blank in a spectrophotometer at 620nm against distilled water
Calculation:

The amylase activity (level) was calculated and expressed in iu/L in the test sample using the following formula:

$$\text{Amylase (iu/L)} = \frac{AB - A\,T \times 800}{AB}$$

Where:
AB = Absorbance of reagent Black
AT = Absorbance of Test.

Preparation of Reagents
The phosphate buffered starch substrate and iodine solution were prepared according to the method described by cheesbrough 1987 [5].

To prepare starch substrate at PH 7.0

Di- sodium hydrogen phosphate 26.000 (anhydrous) Na_2HPO_4

Sodium Chloride	1.75g
Benzoic acid	8.60g
Soluble Starch	0.40g
Distrilled water	to 1 litre

Procedure:
Weigh the di-sodium hydrogen phosphate, sodium chloride and Benzoic acid. Transfer these chemicals to a heat resistant beaker or flask.

❖Add about 500ml of water and mix. Place the beaker or flask in a container of water and heat to boiling. Mix at intervals to dissolve the chemicals.
❖Weight the starch and dissolve it in about 10ml of cold distilled water.
❖With mixing stir the starch suspension to the hot solution in the beaker. Continue mixing until the solution reaches 100°c Allow to boil for 1 minute.
❖Cool to room temperature. Transfer to a 1 litre volulumetric flask and make up to the litre mark with water. Mix well.
❖Transfer to a clean leak proof bottle and label. Store at room temperature. The reagent is stable for about 1 year.

Warming Solution:
This is prepared by mixing 1ml of stock iodine solution with 9ml of distilled water.

RESULTS

Tables 1, 2 and 3 showed the results obtained for the serum, plasma and urine amylase activities (levels) in typhoid fever, HIV infected and normal subjects.

Table 1: Mean values for serum amylase activity for typhoid infected, HIV patients and normal subjects.

Study Population	Mean Valves	Normal Reference Ranges
Typhoid Patients	149 ± 17.92 iu/L	62 – 220 iu/L
HIV Patients	210 ± 10. 96 iu/L	180 – 231 iu/L
Normal Subjects	106 ± 5.99 iu/L	73 - 135 iu/L

Table 2: Mean Values for plasma Amylase Activity for Typhoid fever, HIV patients and Normal Subjects.

Study Population	Mean Values	Normal Reference Ranges
Typhoid patients	144 ± 9.6 iu/L	62 – 220 iu/L
HIV patients	221 ± 7.88 iu/L	152- 294 iu/L
Normal Subjects	124 ± 4.36 iu/L	73 -178 iu/L

Table 3: Mean Values for Urine Amylase activity of typhoid patients, HIV patients and Normal subjects.

Study population	Mean Values	Normal Reference Ranges
Typhoid patients	199 ± 21.52 iu/L	169 – 220 iu/L
HIV patients	282 ± 17. 0 iu/L	265 – 299 iu/L
Normal Subjects	152 ± 13. 04 iu/L	130 – 180 iu/L

Mean Amylase Activity (iu/L)

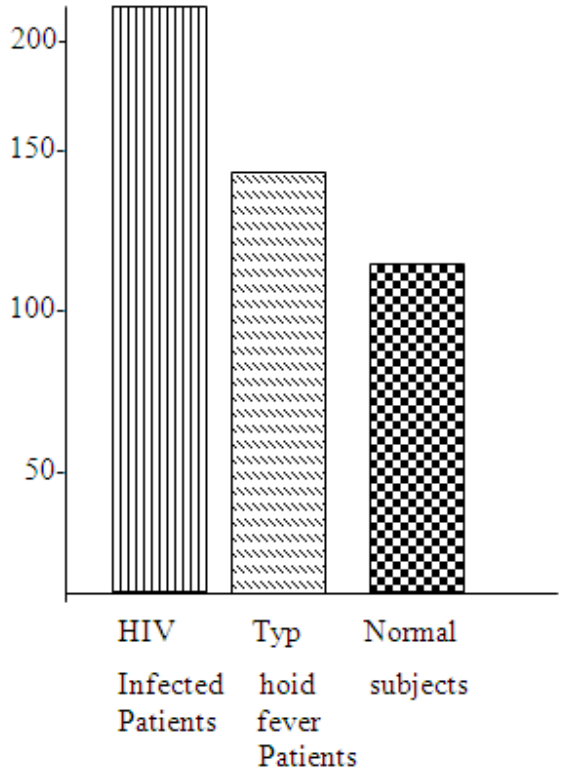

Figure 1: Bar chart showing the mean serum Amylase Activity of typhoid fever patients, HIV infected and normal subjects

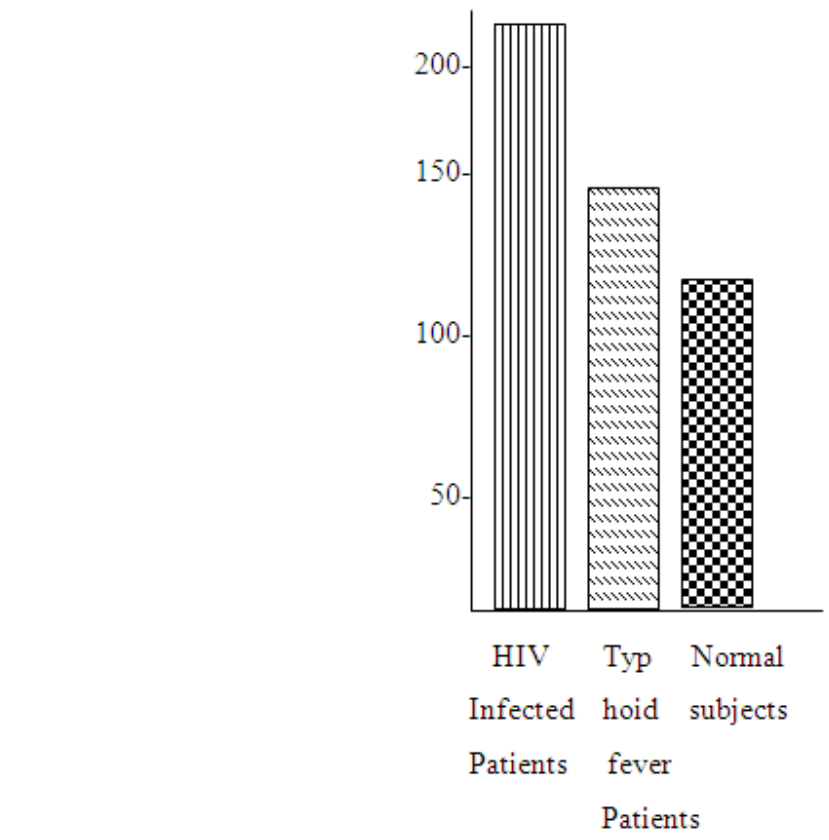

Figure 2: Bar chart showing the mean plasma Amylase Activity of Typhoid fever patients, HIV subject and normal subject

Mean Amylase Activity (iu/L)

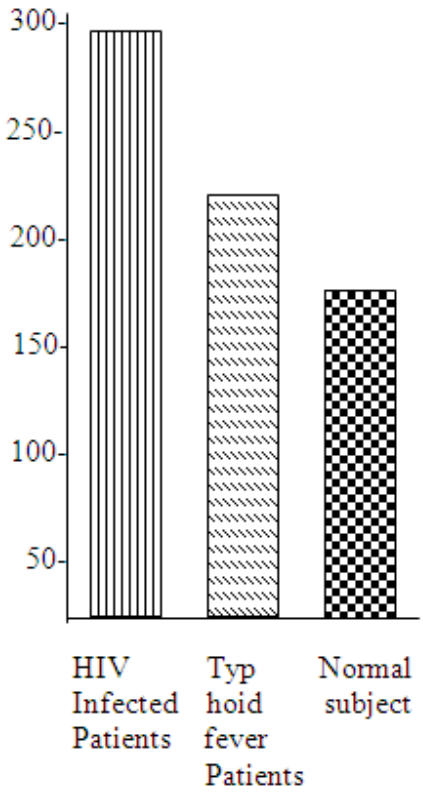

Figure 3: Bar chart showing the mean Urinary Amylase Activity of Typhoid fever patients, HIV infected and Normal subjects

DISCUSSION

A Comparative assessment of the enzymatic activities (levels) of the serum, plasma and Urinary amylase was done on typhoid, HIV and Normal subjects. This was with the aim of establishing if it could be used as a diagnostic tool. Of the 206 subjects used in the study 81 were typhoid fever subjects and 75 were HIV infected patients while 50 normal subjects were used as controls. The study revealed that the mean serum amylase activity for typhoid fever, HIV infected and normal subjects were 149 ± 17.92 iu/L, 210 ± 10 .96 iu/L and 106 ± 5.99 iu/L respectively. The study also revealed that the mean plasma and urinary amylase activity(level) for typhoid fever, HIV infected and normal subjects were 144 ± 9.6 iu/L, 221 ± 7.88 iu/L and 124 ± 17. 0 iu/L and 152 ± 13. 04 iu/L respectively.

Using the student t- test to statistically evaluate the differences in mean amylase activity of the categories of the subjects studied showed that there were significant differences ($p < 0.05$) between them.

The mean serum, plasma and urinary amylase levels of typhoid fever and HIV infected patients were found to be higher when compared with that of the Normal subjects although the various mean amylase levels fell within the normal reference range (70 -340 iu/L) [5]

The appreciable high levels of serum, plasma and urinary amylase activities in typhoid fever and HIV infected subjects could have been higher if these subjects were examined early enough when the serum and urinary amylase activities (levels) could have risen to their peaks. It has however been claimed that the urinary amylase levels may be elevated for 7 to 10 days whereas the rise in serum amylase is often very brief with enzymes reaching its highest

levels within 48 to 72 hours and returning to normal within 48 to 72 hours after an attack, [1, 5].The serum, plasma and amylase activities (levels) in this study fell within the normal range for the following reasons:

- The patients involved have been receiving treatment for a considerably long time; hence their peak amylase values could not be ascertained. This returns to normal 24 to 48 hours and 7 to 10 days after the onset of the illness for serum, plasma and urinary amylase activities respectively.
- The particular stage of HIV infection could not be ascertained. The particular stage of HIV infection may affect the serum or urinary amylase levels which could still progress to higher levels as it has been popularly claimed that the clinical consequences of HIV infection encompass a spectrum ranging from an acute syndrome associated with primary infection to a prolonged asymptomatic stage to an advanced stage, [6, 7]. The fact that levels of serum, plasma and urinary amylase activities fell within the normal range does not rule out the possibility of acute pancreatitis in typhoid fever and HIV infected patients.

It is recognized however that serum amylase activity goes very high in acute pancreatitis and it is observed that certain glands in typhoid and HIV patients especially the pancreas are infected during an attack. This obviously could give rise to high amylase activity / level [8]. From this study it has been shown that either serum or plasma samples could be used to determine amylase activity (level) of a subject. It was confirmed in urine samples than in serum or plasma samples of a subject. It has been claimed that the urinary excretion of amylase is higher in patients with acute pancreatitis.

The increased excretion of amylase persists longer than the elevation in serum amylase activity and can help to establish the diagnosis of acute pancreatitis [1]. In this study also normal subjects were used as controls and their serum, plasma and urinary amylase activities fell within the normal range.

It was not possible to differentiate between HIV and AIDS patients but it assumed that those examined have developed into AIDS since some of them have over a year been visiting the hospital for treatment of their illnesses. Therefore it was not possible to distinguish between HIV and AIDS patients based on their various amylase activities/ levels.

CONCLUSION

The serum plasma and urinary amylase activities/ levels of both typhoid and HIV/ AIDS patients were slightly higher than the normal (control) but both fell within the normal range. The urinary amylase activity of typhoid fever, HIV / AIDS and normal subjects were higher than the serum/ plasma amylase activity of the same subjects also either serum or plasma could be used to determined the amylase activity of subjects. Despite these results the conclusion is that serum, plasma and urinary amylase activities could be used as diagnostic tools to confirm the onset of typhoid fever or HIV disease if these tests are carried out as early as possible since the use of amylase activity is bound to improve the diagnostics efficiency of typhoid fever in particular which hitherto adopts the Widal Test technique.

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